

## PROPOSED CHANGES

June 4, 1996

The final Information Collection Rule, as published on May 14, 1996 (Federal Register **61**, 24354-24388), incorporates the ICR Microbial Laboratory Manual as part of the rule by reference. Procedures in the published version of this manual, dated April, 1996, must be followed until changes are added to the manual by a formal amendment. The following is a list of corrections and modifications that have been proposed. When the manual is amended, you will be informed on this home page.

### Section III

Page 3, paragraph 3: “A set of PE samples for ongoing approval will have two samples” should now read “A set of PE samples for ongoing approval will consist of two samples per month.”

Page 4, item 2b: “10,000 MPN units per 100 L (approximately 100 infectious virus particles per liter)” should read “1,000 MPN units per 100 L (10 MPN units per liter).”

### Section VII

Page 24, step 2: “Pipet 1.0 ml” should read “Pipet 0.5 ml.”

Page 24, step 5: “Pipet 1.0 ml” should read “Pipet 0.5 ml.”

Page 24, step 9: “95% ethanol solutions” should read “90.2% ethanol solutions.”

### Section VIII

Page 12, step 1: “3-3 min” should read “2-3 min.”

Page 19, steps 8-11: replace with the following:

“Step 8. Turn off the pressure at the source and open the vent/relief valve on the pressure vessel. Thoroughly mix the eluate.

- a. If archiving is not required and if the optional coliphage assay is not performed, measure the volume of the eluate and record it onto the **Virus Data Sheet** as the **Eluate Volume Recovered**. Take the **Total Sample Volume** value from the **Sample Data Sheet** and record it as the **Adjusted Total Sample Volume** on the **Virus Data Sheet**.
- b. If archiving is required or if the optional coliphage assay (see **Section IX. Coliphage Assay**) will be performed, adjust the pH of the eluate to 7.0-7.5 with 1 M HCl. Measure the volume of the adjusted eluate and record it onto the **Virus Data Sheet** as the **Eluate Volume Recovered**.

- i. Determine the amount of sample to be removed for archiving by multiplying the **Eluate Volume Recovered** by 0.1. Record this amount onto the **Virus Data Sheet** as the **Volume of Eluate Archived** and place it into a separate container. Freeze<sup>3</sup> the archive sample and ship it to the ICR Laboratory Coordinator, USEPA, TSD, 26 W. Martin Luther King Drive, Cincinnati, OH 45268.
- ii. Determine the amount of sample to be used in the coliphage assay by multiplying the **Eluate Volume Recovered** by 0.035. Place a volume equal to this amount into a separate container and store at 4°C.
- iii. Multiply the recorded **Total Sample Volume** from the **Sample Data Sheet** by 0.9 if an archive sample is taken, by 0.965 if a coliphage sample is taken or by 0.865 if both an archive sample and a coliphage sample are taken. Record the amount on the **Virus Data Sheet** as the **Adjusted Total Sample Volume**.

Step 9. Proceed to the **Organic Flocculation Concentration Procedure** immediately. If the **Organic Flocculation Concentration Procedure** cannot be undertaken immediately, store the eluate (adjusted to pH 7.0 to 7.5 as described in Step 8b) at 4°C for up to 24 h or for longer periods at -70°C.”

Page 24, step 3, change to read:

“Step 3. Determine the **Inoculum Volume** by dividing the **Assay Sample Volume** by 20. Record the **Inoculum Volume** onto the **Virus Data Sheet**.

*For ease of inoculation, a sufficient quantity of 0.15 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.0 - 7.5, may be added to the **Inoculum Volume** to give a more usable working **Inoculation Volume** (e.g., 1.0 mL). For example, if an **Inoculum Volume** of 0.73 mL is to be placed onto 10 vessels, then  $10.5 \times (1 - 0.73 \text{ mL}) = 2.84 \text{ mL}$  of sodium phosphate, pH 7.0-7.5 could be added to  $10.5 \times 0.73 = 7.67 \text{ mL}$  of subsample. Each milliliter of the resulting mixture will contain the required **Inoculum Volume**.*

*The **Inoculum/Inoculation Volume** should be no greater than 0.04 mL/cm<sup>2</sup> of surface area. If the **Inoculum** or **Inoculation Volume** is greater than 0.04 mL/cm<sup>2</sup>, use larger culture vessels.”*

Page 24, step 4: change to read:

“Step 4. Inoculate each BGM cell culture test vessel with an amount of assay control or water sample equal to the **Inoculum** or **Inoculation Volume** and record the date of inoculation on the **Sample Data Sheet** (see **Part 9**).

*Avoid touching either the cannula or the pipetting device to the inside rim of the cell culture test vessels to avert the possibility of transporting contaminants to the remaining culture vessels.”*

Page 24, step 4ai and 4aii: where “**Inoculation Volume**” is used, it should now read “**Inoculum** or **Inoculation Volume**.”

Page 25, step 4bi: where “**Inoculum Volume**” is used, it should now read “**Inoculum or Inoculation Volume**.”

Page 25, step 4bii: following the sentence, “After mixing thoroughly, prepare a 1:25 dilution by adding amount “a” of the 1:5 diluted sample to amount “b” of 0.15 M sodium phosphate (pH 7.0-7.5),” insert “A working **Inoculation Volume** may be prepared from undiluted subsample 2 and from subsample 2 diluted 1:5 and 1:25 as described in Step 3.” Where “**Inoculum Volume**” is used, it should read “appropriate **Inoculum or Inoculation Volume**.”

Page 26, step 4c: “**Inoculum Volume**” should read “**Inoculum or Inoculation Volume**.”

Page 27, step 10: “**Inoculum Volume**” should read “**Inoculum or Inoculation Volume**.”

Page 49, 2nd paragraph: “**inoculation volume**” in the 1st sentence should read “**Inoculum Volume**.” “**inoculum volume**” in the 2nd sentence should read “**Inoculum Volume**.” “200 PFU/mL” in the 6th sentence should read “20 PFU/ mL.”

Pages 53, 60 and 65, Virus Data Sheet: “VOLUME OF ORIGINAL WATER SAMPLE ASSAYED (D):” should read “VOLUME OF ORIGINAL WATER SAMPLE ASSAYED (D):<sup>2</sup>.”

Page 56, 4th paragraph: “**inoculation volume**” in the 1st sentence should read “**Inoculum Volume**.” “241.0 PFU/mL (200 PFU/0.82 ml)” in the 3rd sentence should read “25 PFU/mL (20 PFU/0.82 mL).”

## **Appendix B**

Page 2, last paragraph: “used initially and periodically thereafter to demonstrate” in the 2nd sentence should read “used to demonstrate.”

Page 9, last paragraph: “used initially and periodically thereafter to demonstrate” in the 2nd sentence should read “used to demonstrate.”

## **Appendix D**

Page 11, item 6.1: “dated July, 1995” should read “dated April, 1996.”

### **NOTICE:**

The corrections and modifications listed above are presented for your information only. They will not become part of the Information Collection Rule until official steps are taken to amend the rule. Additional items may be added to this list as they are identified.